Data Evaluation Record on the Acute Toxicity of GF-2726 (2,4-D Choline Salt + Glyphosate DMA) to Terrestrial Vascular Plants: Seedling Emergence

PMRA Submission Number {.....} **EPA MRID Number 49903203**

Data Requirement:
PMRA Data Code: 9.8.4 (TGAI) or 9.8.6 (EP)
EPA DP Barcode:
OECD Data Point: IIA 8.12 (TGAI) and IIIA 10.8.1.1 (EP)
EPA Guideline: 850.4100

Test material: **2,4-D Choline Salt** **Purity:** 24.1%
Glyphosate Dimethylammonium **Purity:** 21.7%
Common name:
Chemical name: IUPAC:
CAS name:
CAS No.:
Synonyms: GF-2726, TSN306327

Primary Reviewer: Elizabeth Krupka
Senior Scientist, CDM Smith
Signature:
Date: 6/14/16

Secondary Reviewer: Teri S. Myers
Senior Scientist, CDM Smith
Signature:
Date: 6/22/16

Primary Reviewer: Edward Odenkirchen, Ph.D./EPA
{EPA/OECD/PMRA}
Date: 08/02/2016

Secondary Reviewer(s): Kristina Garber/EPA
{EPA/OECD/PMRA}
Date: 08/11/2016

Reference/Submission No.: 08/12/2016

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
Use Site Category: {.....} [For PMRA]
EPA PC Code: **051505 (2,4-D Choline Salt)**
103608 (Glyphosate DMA)

Date Evaluation Completed: 08/12/2016

CITATION: Bergfield, A. 2016. GF-2726 (2,4-D Choline Salt, 286 g a.s/L; Glyphosate Dimethylammonium 260 g a.s./L; SL): Effects on the Seedling Emergence and Growth of Non-Target Terrestrial Plants - Non-Crop Species (Tier II). Unpublished study performed by ABC Laboratories, Columbia, Missouri, and sponsored by Dow AgroSciences LLC, Indianapolis, Indiana. ABC Study No. 83627; Dow AgroSciences Study No. 160374. Study completed April 29, 2016.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to terrestrial vascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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Data Evaluation Record on the Acute Toxicity of GF-2726 (2,4-D Choline Salt + Glyphosate DMA) to Terrestrial Vascular Plants: Seedling Emergence

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EXECUTIVE SUMMARY:

The effect of **GF-2726 (2,4-D Choline Salt + Glyphosate Dimethylammonium)** on the seedling emergence of the dicot (Lambsquarters, *Chenopodium album*) was studied. This species is a target of the formulation tested (i.e. it is considered a weed species). Nominal concentrations were 0 (negative control), 0.00138, 0.0027, 0.0055, 0.0110, 0.0221, 0.044, 0.088, 0.176, 0.35, 0.71, and 1.41 lb 2,4-D Choline Salt/A, and were 0 (negative control), 0.00124, 0.0025, 0.0050, 0.0099, 0.0199, 0.040, 0.079, 0.159, 0.32, 0.64, and 1.27 lb Glyphosate DMA/A. Measured concentrations, used in analyses for the three highest treatment levels were 0.36, 0.72, and 1.42 lb 2,4-D Choline Salt/A. Glyphosate DMA concentrations were not confirmed analytically.

The growth medium used in the seedling emergence test was a top soil silica sand mix (sandy loam, pH 6.4, organic carbon 1.5%). On day 28 the surviving plants per pot were recorded and cut at soil level for measuring the plant height and dry weight.

Seedling emergence in the negative control was 43%. The reviewer found no significant inhibitions in emergence compared to the negative control. The reviewer based survival on the number planted, and survival in the negative control was 41%. The reviewer found no significant inhibitions in survival.

The reviewer found significant inhibitions in seedling height compared to the negative control of 39, 38, 49, and 52% at the 0.176, 0.36, 0.72, and 1.42 lb 2,4-D Choline Salt/A treatment levels, respectively (Jonckheere-Terpstra test, $p<0.05$).

The reviewer found significant inhibitions in dry weight in compared to the negative control of 61, 62, 84, and 67% at the 0.176, 0.36, 0.72, and 1.42 lb 2,4-D Choline Salt/A treatment levels, respectively (Jonckheere-Terpstra test, $p<0.05$).

The most sensitive endpoint was dry weight, with NOAEC and IC_{25} values of 0.088 and 0.101 lb 2,4-D Choline Salt/A, respectively.

In terms of Glyphosate DMA, the most sensitive endpoint was dry weight with NOAEC and IC_{25} values of 0.0792 and 0.0909 lb Glyphosate DMA/A, respectively. In terms of Total Product GF-2726, the most sensitive endpoint was dry weight with NOAEC and IC_{25} values of 0.37 and 0.419 lb Total Product/A, respectively.

There were none to moderate phytotoxic effects (0-64) in lambsquarters that appeared to be dose related.

Maximum Labeled Rate: Not reported

Results Synopsis

2,4-D Choline Salt

Most sensitive endpoint: dry weight

EC_{50}/IC_{50}: 0.392 lb ai/A	95% C.I.: 0.221-0.777 lb ai/A
EC_{25}/IC_{25}: 0.101 lb ai/A	95% C.I.: 0.0482-0.205 lb ai/A
EC_{05}/IC_{05}: 0.0328 lb ai/A	95% C.I.: 0.00899-0.0815 lb ai/A
NOEC: 0.088 lb ai/A	
Slope: NC	95% C.I.: N/A

Glyphosate DMA

Most sensitive endpoint: dry weight

EC_{50}/IC_{50}: 0.353 lb ai/A	95% C.I.: 0.199-0.700 lb ai/A
EC_{25}/IC_{25}: 0.0909 lb ai/A	95% C.I.: 0.0434-0.185 lb ai/A
EC_{05}/IC_{05}: 0.0295 lb ai/A	95% C.I.: 0.00809-0.0734 lb ai/A
NOEC: 0.0792 lb ai/A	
Slope: NC	95% C.I.: N/A

Table 1 (Tier II studies). Summary of most sensitive parameters by species (lb 2,4-D Choline Salt/A).

Species	Endpoint	NOEC	EC_{05}/IC_{05}	EC_{25}/IC_{25}	EC_{50}/IC_{50}
Lambsquarters#	Dry weight	0.088	0.0328	0.101	0.392

Jonckheere-Terpstra Step-Down test run for trends analysis; CETIS was unable to run the Williams test on more than 10 treatment levels.

Table 1a (Tier II studies). Summary of most sensitive parameters by species (lb Glyphosate DMA/A).

Species	Endpoint	NOEC	EC_{05}/IC_{05}	EC_{25}/IC_{25}	EC_{50}/IC_{50}
Lambsquarters#	Dry weight	0.0792	0.0295	0.0909	0.353

Jonckheere-Terpstra Step-Down test run for trends analysis; CETIS was unable to run the Williams test on more than 10 treatment levels.

This study is scientifically sound and is classified as acceptable.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in compliance with OCSPP Guideline 850.4100: Seedling Emergence and Seedling Growth (January 2012). The reviewer evaluated the study methods according to EPA Ecological Effects Test Guidelines, OCSPP Guideline 850.4100: Seedling Emergence and Seedling Growth. There were some deficiency and deviations noted by the reviewer.

1. The physico-chemical properties of the test material were not reported.

2. Mean emergence in the control was only 43%, and significant variability was observed between replicates, with CVs for emergence of up to 89%. USEPA guidance standards indicate that the test is not acceptable if less than 70% of control plants emerged. EPA waived the standard emergence requirement for this special study. The species used was by EPA request and the seed germination rate is an expected limitation.

The deficiency and deviations did not have an impact on the acceptability of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted in compliance with USEPA Good Laboratory Practice Standards (40 CFR, Part 160, 1989), with the following exceptions: the latest water characterizations performed in June 2015, and the photographic data of test plants, were not collected in accordance with the stated GLP.

A. MATERIALS:

1. Test Material **GF-2726 (2,4-D Choline Salt + Glyphosate Dimethylammonium)**

Description: Solid

Lot No./Batch No.: 2C01163R01

Purity: 2,4-D Choline Salt: 24.1%
Glyphosate DMA: 21.7%

Stability of compound under test conditions: Analytical determinations for lambsquarters based on measured concentration of the three highest test concentrations in the initial spray solution yielded recoveries of 101-103% of nominal (n = 6). Analytical determinations based on measured concentration of the three highest test concentrations in the post application spray solution yielded recoveries of 100-102% of nominal (n = 6). The comparability between pre- and post-application spray solutions indicates the test substance was stable over the treatment period.

(OECD recommends chemical stability in water and light)

Storage conditions of test chemicals: The test material was stored at room temperature.

Table 2. Physical/chemical properties of GF-2726.

Parameter	Values	Comments
Water solubility at 20˚C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Monocotyledonous species: None; *EPA recommends four monocots in two families, including corn.*

Dicotyledonous species: Lambsquarters (*Chenopodium album*, Chenopodiaceae); *EPA recommends six dicots in four families, including soybean and a root crop.*

OECD recommends a minimum of three species selected for testing, at least one from each of the following categories: Category 1: ryegrass, rice, oat, wheat, and sorghum; Category 2: mustard, rape, radish, turnip, and Chinese cabbage; Category 3: vetch, mung bean, red clover, fenugreek, lettuce, and cress.

Seed source: Lambsquarters obtained from Dow AgroSciences.
Prior seed treatment/sterilization: The seeds were not treated with any type of fungicides, insecticides, or any pesticides.
Historical % germination of seed: Lambsquarters, 75%.
Seed storage, if any: Not reported.

B. STUDY DESIGN:

1. Experimental Conditions

a. Limit test: None.

b. Range-finding study: None.

c. Definitive Study

Table 3: Experimental Parameters - Seedling Emergence.

Parameters	Seedling Emergence	
	Details	**Remarks** ***Criteria***
Duration of the test	28 days	*Recommended test duration is 14-21 days.* *OECD recommends that the test be terminated no sooner than 14 days after 50 percent of the control seedlings have emerged*
Number of seeds/plants/species/ replicate	7 seeds per pot.	*Ten seeds per replicate should be used.* *OECD recommends a minimum of five seeds planted in each replicate within 24 hours of incorporation of the test substance. All seeds of each species for each test should be of the same size class. The seed should not be imbibed.*
Number of replicates Control: Adjuvant control: Treated:	6 N/A 6	*Four replicates per dose should be used.* *OECD recommends a minimum of four replicates per treatment*
Test concentrations (lb ai/A) Nominal: Measured:	0 (negative control), 0.00138, 0.0027, 0.0055, 0.0110, 0.0221, 0.044, 0.088, 0.176, 0.35, 0.71, and 1.41 lb 2,4-D Choline Salt/A Three highest test concentrations: 0.36, 0.72, and 1.42 lb 2,4-D Choline Salt/A.	Nominal concentrations for Glyphosate DMA: 0 (negative control), 0.00124, 0.0025, 0.0050, 0.0099, 0.0199, 0.040, 0.079, 0.159, 0.32, 0.64, and 1.27 lb Glyphosate DMA/A Nominal concentrations for GF-2726 product: 0.00572, 0.0114, 0.0229, 0.0457, 0.0915, 0.183, 0.366, 0.732, 1.46, 2.93, and 5.85 lb product/A. *Five test concentrations should be used with a dose range of 2X or 3X progression* *OECD recommends three concentrations, preferably with application rates equivalent to 0.0 (control), 1.0, 10.0 and 100 mg substance per kg of oven-dried soil.*
Method and interval of analytical verification	Spray solutions were analyzed by HPLC using a Waters Symmetry	2,4-D acid, the acid equivalent active ingredient of GF-2726, was measured in the

Parameters	Seedling Emergence	
	Details	**Remarks** ***Criteria***
LOQ: LOD:	C18 column. 0.0184 lb 2,4-D Choline Salt/A (MQL) Not reported.	spray solutions, and converted to 2,4-D Choline Salt concentrations.
Adjuvant (type, percentage, if used)	N/A	
<u>Test container (pot)</u> Size/Volume Material: (glass/polystyrene)	Pots with top diameter of 16.5 cm diameter x 11.5 cm depth. Plastic	*Non-porous containers should be used.* *OECD recommends that non-porous plastic or glazed pot be used.*
Growth facility	Greenhouse	
Method/depth of seeding	Lambsquarters planted at a depth of 6 mm.	
<u>Test material application</u> Application time including the plant growth stage Number of applications Application interval Method of application	After planting 1 N/A- single application Application of the test substance was made using an overhead track sprayer (De Vries) equipped with a TeeJet 4002E nozzle operated at 40 psi, approximately 27 inches above the soil surface (140 L/ha nominal spray volume)	
<u>Details of soil used</u> Geographic location Depth of soil collection	Lime Spring, Iowa N/A	Top soil mixed with silica sand. Organic Matter: 2.5%

Parameters	Seedling Emergence	
	Details	**Remarks**
		Criteria
Soil texture % sand % silt % clay pH: % organic carbon CEC Moisture at 1/3 atm (%)	Sandy loam 71 18 11 6.4 1.5% 11.0 meq/100g 13.9%	*Soil mixes containing sandy loam, loam, or clay loam soil with no greater than 2% organic matter are preferable. Glass beads, rock wool, and 100% acid washed sand are not preferred.* *OECD prefers the soil to be sieved (0.5 cm) to remove coarse fragments. Carbon content should not exceed 1.5% (3% organic matter). Fine particles (under 20um) makeup should be between 10 and 20%. The recommended pH is between 5.0 and 7.5.*
Details of nutrient medium, if used	Peter's 20-20-20 (1/2 tablespoon/gallon). Applied once via sub-irrigation to all species.	
<u>Watering regime and schedules</u> Water source/type: Volume applied: Interval of application: Method of application:	Top watered once post-application, then sub-irrigation. Well water. Not reported. Daily. The plants were bottom watered daily as needed.	*EPA prefers that bottom watering be utilized for seedling emergence studies so that the chemical is not leached out of the soil during the test.*
Any pest control method/fertilization, if used	None reported.	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality: Relative humidity:	 mean 21.9, range 13.9-28.1˚C 16L:8D Natural sunlight supplemented with artificial light. mean 244, range 142-359 PAR ($\mu mol/m^2/sec$) Mean: 78%, range 15-93%	*EPA prefers that the cold vs warm loving plants be tested in two separate groups to optimize plant growth.* *OECD prefers that the temperature, humidity and light conditions be suitable for maintaining normal growth of each species for the test period.*

Parameters	Seedling Emergence	
	Details	**Remarks** ***Criteria***
Reference chemical (if used) Name: Concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 4: Observation Parameters - Seedling Emergence.

Parameters	Seedling Emergence	
	Details	**Remarks**
Parameters measured (e.g., number of germinated seeds, emerged seedlings, plant height, dry weight or other endpoints)	- Emergence - Survival - Shoot height - Total dry weight - Phytotoxicity	
Measurement technique for each parameter	Emergence and phytotoxicity were visually determined. Survival was defined as the percent of emerged. Height was measured from the base of the stem to the tip of the longest leaf or apical bud. Total replicate weight was determined following drying.	
Observation intervals	Each pot was inspected weekly, emergence and survival determined. Dry weight and shoot height were recorded at study termination.	
Other observations, if any	N/A	
Were raw data included?	Yes	

Phytotoxicity rating system, if used	No effect, 1-10, no effect; 20-30, slight effect; 40-60, moderate effect; 70-100, severe effect; 100, complete effect.	Frans, R.E. and R.E. Talbert, 1977.

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

1. Seedling Emergence:

Seedling emergence in the negative control was 43%. The study author observed a significant inhibition in seedling emergence at 1.41 lb 2,4-D Choline Salt/A (Cochran-Armitage test, $p<0.05$). The reviewer found no significant inhibitions in emergence compared to the negative control.

Study author survival was based on the number emerged, and in the negative control, survival was 96%. The study author reported no significant inhibitions for lambsquarters survival. The reviewer based survival on the number planted, and survival in the negative control was 41%. The reviewer found no significant inhibitions in survival.

The study author found significant inhibitions in seedling height compared to the negative control of 39, 38, 49, and 52% at the 0.176, 0.35, 0.71, and 1.41 lb 2,4-D Choline Salt/A treatment levels, respectively (Jonckheere-Terpstra test, $p<0.05$). The reviewer found significant inhibitions in seedling height compared to the negative control of 39, 38, 49, and 52% at the 0.176, 0.36, 0.72, and 1.42 lb 2,4-D Choline Salt/A treatment levels, respectively (Jonckheere-Terpstra test, $p<0.05$).

The study author found significant inhibitions in lambsquarters dry weight compared to the negative control of 54, 61, 72, 87, and 84% at the 0.88, 0.176, 0.35, 0.71, and 1.41 lb 2,4-D Choline Salt/A treatment levels, respectively (Jonckheere-Terpstra test, $p<0.05$). The reviewer found significant inhibitions in lambsquarters dry weight were 61, 62, 84, and 67% at the 0.176, 0.36, 0.72, and 1.42 lb 2,4-D Choline Salt/A treatment levels, respectively (Jonckheere-Terpstra test, $p<0.05$).

Based on the study author's results, the most sensitive monocot was not evaluated in this study; the most sensitive dicot was lambsquarters based on dry weight, with NOEC and ER_{25} values of 0.0441 and 0.0453 lb 2,4-D Choline Salt/A, respectively.

There were none to moderate phytotoxic effects (0-64) in lambsquarters that appeared to be dose related.

B. REPORTED STATISTICS:

Emergence, survival, replicate shoot dry weight, and height mean and standard deviations were determined. Statistical analysis of rate versus effect data was performed using SAS Version 9.3. Emergence and survival data were tested using a combination of Fisher's Exact Comparison with Bonferroni-Holm Adjustment, and Cochran Armitage test. Length and weight data sets were tested for normality (Shapiro-Wilk) and homogeneity of variance (Levene). Non-normal and/or non-homogeneous data sets were analyzed using non-parametric procedures (Wilcoxon scores analyzed using Dunn's multiple comparison), as well as trend testing (Jonckheere's). Normally distributed and homogenous data sets were analyzed using parametric procedures (Dunnett's pair-wise comparison), as well as trend testing (Jonckheere's). All statistical determinations were made with 95% certainty. Due to significant effects from NOER determinations, emergence and post-emergent

survival data were analyzed using Probit methods, and plant shoot length and dry weight data was analyzed using non-linear regression dose-response models (Bruce, Versteeg Weighted Probit, Schabenberger Hormetic, and OECD Model 2; all models were fitted to the data using the Marquardt method). Nominal concentrations were used for all analyses.

Table 5: Effect of GF-2726 on 28-Day Seedling Emergence

Species	**Results summary for height (lbs 2,4-D Choline Salt/A)**									
	height (mm)	NOEC	IC_{05}	95%CI	IC_{25}	95%CI	IC_{50}	95%CI	slope	95%CI
Lambsquarters[1]	47.8-145	0.0882	ND	N/A	0.134	N/A	0.648	N/A	ND	N/A

ND- Not determined. N/A- Not applicable.
1 Significant decrease in lambsquarter height of 39, 38, 49 and 52% at 0.176, 0.35, 0.71 and 1.41 lb ai/A (Jonckheere-Terpstra, $p<0.05$).

Table 5a: Effect of GF-2726 on 28-Day Seedling Emergence

Species	**Results summary for biomass (lbs 2,4-D Choline Salt/A)**									
	Weight (g)	NOEC	IC_{05}	95%CI	IC_{25}	95%CI	IC_{50}	95%CI	slope	95%CI
Lambsquarters[1]	0.0812-0.783	0.0441	ND	N/A	0.0453	N/A	0.109	N/A	ND	N/A

ND- Not determined. N/A- Not applicable.
1 Significant decrease in lambsquarter weight of 54, 61, 72, 87 and 84% at 0.088, 0.176, 0.35, 0.71 and 1.41 lb ai/A (Jonckheere-Terpstra, $p<0.05$).

Table 5b: Effect of GF-2726 on 28-Day Seedling Emergence

Species	**Results summary for emergence (lbs 2,4-D Choline Salt/A)**									
	%	NOEC	EC_{05}	95%CI	EC_{25}	95%CI	EC_{50}	95%CI	slope	95%CI
Lambsquarters[1]	14-55	0.71	ND	N/A	0.067	N/A	0.786	N/A	ND	N/A

ND- Not determined. N/A- Not applicable.
1 Significant decrease in lambsquarter emergence at 1.41 lb ai/A (Cochran-Armitage, $p<0.05$).

Table 5c: Effect of GF-2726 on 28-Day Seedling Emergence

Species	**Results summary for survival (lbs 2,4-D Choline Salt/A); based on # emerged**									
	%	NOEC	EC_{05}	95%CI	EC_{25}	95%CI	EC_{50}	95%CI	slope	95%CI
Lambsquarters	83-100	1.41	ND	N/A	>1.41	N/A	>1.41	N/A	ND	N/A

ND- Not determined. N/A- Not applicable.

Mid-study emergence	
Control	Lambsquarters
ND	ND

Formula Blank is N/A.

Plant Injury Index*	
Control	Lambsquarters
0	0-64

Formula Blank is N/A.
*1-10 = no effect; 20-30 = slight effect; 40-60 = moderate effect; 70-100 = severe effect; 100 = complete effect.

C. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

All analyses were conducted comparing treated to the negative control. These analyses were conducted using CETIS version 1.8.7.12 and backend settings approved for use by EFED on 10/20/2015. Data for each endpoint were tested to determine if their distributions were normal and if their variances were homogeneous using Shapiro-Wilk's and Levene's tests, respectively. Data that satisfied these assumptions were subjected to Dunnett's and William's tests, and data that did not satisfy these assumptions were subjected to the non-parametric Mann-Whitney U and Jonckheere's tests. Measured concentrations were used in analyses for the three highest treatment levels, and nominal concentrations were used for the lower treatment levels. Linear (survival and emergence) and nonlinear (height and dry weight) regression models were used to interpret EC/ICx values.

Table 6: Effect of GF-2726 on 28-Day Seedling Emergence

Species	Results summary for height (lbs 2,4-D Choline Salt/A)									
	height (mm)	NOEC	IC_{05}	95%CI	IC_{25}	95%CI	IC_{50}	95%CI	slope	95%CI
Lambsquarters[1#]	47.8-145	0.088	NC	N/A	NC	N/A	NC	N/A	NA	N/A

NC- Not calculable. N/A – not applicable

Jonckheere-Terpstra Step-Down test selected for trends analysis; CETIS was unable to run the Williams test on more than 10 treatment levels.

[1] Significant decrease in lambsquarters height, inhibition of 39, 38, 49 and 52% at the 0.176, 0.36, 0.72 and 1.42 lb ai/A treatment levels compared to the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$).

Table 6a: Effect of GF-2726 on 28-Day Seedling Emergence

Species	Results summary for biomass (lbs 2,4-D Choline Salt/A)									
	Weight (g)	NOEC	IC_{05}	95%CI	IC_{25}	95%CI	IC_{50}	95%CI	slope	95%CI
Lambsquarters[1#]	0.0656-0.326	0.088	0.00328	0.00899-0.0815	0.101	0.0482-0.205	0.392	0.221-0.777	NA	N/A

NC- Not calculable. N/A – not applicable

Jonckheere-Terpstra Step-Down test selected for trends analysis; CETIS was unable to run the Williams test on more than 10 treatment levels.

[1] Significant decrease in lambsquarters dry weight, inhibition of 61, 62, 84 and 67% at the 0.176, 0.36, 0.72 and 1.42 lb ai/A treatment levels, respectively, compared to the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$).

Table 6b: Effect of GF-2726 on 28-Day Seedling Emergence

Species	Results summary for emergence (lbs 2,4-D Choline Salt/A)									
	%	NOEC	EC_{05}	95%CI	EC_{25}	95%CI	EC_{50}	95%CI	slope	95%CI
Lambsquarters*	14-55	1.42	NC	N/A	0.0000001	N/A-2.26E-05	0.000578	N/A-0.00465	0.182	0.0585-0.305

NC- Not calculable. N/A – not applicable

*Endpoints and/or confidence intervals are outside the tested range of concentrations and should be interpreted with caution. Although there were inhibitions of 39, 33, and 67% at 0.088, 0.36, and 1.42 lb ai/A treatment level, respectively, the corresponding CVs were 64, 71, 52 and 89% and the effects were not significant.

Table 6c: Effect of GF-2726 on 28-Day Seedling Emergence

Species	Results summary for survival (lbs 2,4-D Choline Salt/A); based on # planted									
	%	NOEC	EC_{05}	95%CI	EC_{25}	95%CI	EC_{50}	95%CI	slope	95%CI
Lambsquarters*	14-55	1.42	NC	N/A	0.0000000	N/A-0.000019	0.00046	N/A-0.0041	0.179	0.055-0.302

NC- Not calculable. N/A – not applicable

*Endpoints and/or confidence intervals are outside the tested range of concentrations and should be interpreted with caution. Although there were inhibitions of 35, 29 and 65% at 0.088, 0.36 and 1.42 lb ai/A treatment level, the corresponding CVs were 64, 71, and 89% and the effects were not significant.

Mid-study emergence	
Control	Lambsquarters
ND	ND

Formula Blank is N/A.

Plant Injury Index*	
Control	Lambsquarters
0	0-64

Formula Blank is N/A.

*1-10 = no effect; 20-30 = slight effect; 40-60 = moderate effect; 70-100 = severe effect; 100 = complete effect.

Most sensitive endpoint: dry weight

EC_{50}/IC_{50}: 0.392 lb ai/A 95% C.I.: 0.221-0.777 lb ai/A
EC_{25}/IC_{25}: 0.101 lb ai/A 95% C.I.: 0.0482-0.205 lb ai/A
EC_{05}/IC_{05}: 0.0328 lb ai/A 95% C.I.: 0.00899-0.0815 lb ai/A
NOEC: 0.088 lb ai/A
Slope: NC 95% C.I.: N/A

Most sensitive endpoint: dry weight

EC_{50}/IC_{50}: 0.353 lb ai/A 95% C.I.: 0.199-0.700 lb ai/A
EC_{25}/IC_{25}: 0.0909 lb ai/A 95% C.I.: 0.0434-0.185 lb ai/A
EC_{05}/IC_{05}: 0.0295 lb ai/A 95% C.I.: 0.00809-0.0734 lb ai/A

NOEC: 0.0792 lb ai/A
Slope: NC 95% C.I.: N/A

D. STUDY DEFICIENCIES:

1. The physico-chemical properties of the test material were not reported.

   Mean emergence in the control was only 43%, and significant variability was observed between replicates, with CVs for emergence of up to 89%. USEPA guidance standards indicate that the test is not acceptable if less than 70% of control plants emerged. This weed species is not routinely used for these standardized toxicity tests. The species has a high expected natural rate of nonviable seed.

E. REVIEWER'S COMMENTS:

The species was selected for this study based on results of field testing for U.S. Patent applications that indicated that the Enlist Duo active ingredients combined had the potential to produce toxicity in excess of that predicted by simple effect addition of the individual active ingredients. USEPA, in agreement with the registrant, accepted the limitations of testing this weed species under the guideline study conditions so as to have a data set that was comparable to the field testing results for potential excess toxicity and provided risk assessors with effects endpoints consistent with risk assessment methods.

The reviewer and study author results were similar. Based on the study author's results, the most sensitive endpoint was lambsquarters dry weight, with NOEC and ER_{25} values of 0.0441 and 0.0453 lb 2,4-D Choline Salt/A, respectively. The reviewer determined that the most sensitive endpoint was lambsquarters dry weight, with NOEC and IC_{25} values of 0.088 and 0.101 lb 2,4-D Choline Salt/A, respectively. The reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

The reviewer selected the Jonckheere-Terpstra Step-Down test for trends analysis for lambsquarters height and dry weight because CETIS was not able to run the Williams test on more than 10 treatment levels; the Williams test may have been more appropriate for the data.

The in-life portion of this study was February 19, 2016 to March 18, 2016.

F. CONCLUSIONS:

This study is scientifically sound and is classified acceptable. The most sensitive endpoints for lambsquarters were NOAEC and IC_{25} values of 0.088 and 0.101 lb 2,4-D Choline Salt/A, respectively.

III. REFERENCES:

1. U.S. Environmental Protection Agency - 1982. Pesticide Assessment Guidelines. Subdivision J. Hazard Evaluation: Non-Target Plants; Series 123-1 Seed germination/seedling emergence and vegetative vigor (Tier 2).

2. U.S. Environmental Protection Agency, Series 850- Ecological Effects Test Guidelines, OCSPP Number 850.4100: Seedling Emergence and Seedling Growth. 2012.

3. Frans, R.E. and Talbert, R.E., Design of Field Experiments and the Measurement and Analysis of Plant Responses. Pages 15-23 in B. Truelove, ed. Research Methods in Weed Science. Southern Weed Science Society, Auburn University, Alabama, 1977.

4. Bergfield, A. 2016. GF-2726 (2,4-D Choline Salt, 286 g a.s/L; Glyphosate Dimethylammonium 260 g a.s./L; SL): Effects on the Seedling Emergence and Growth of Non-Target Terrestrial Plants (Tier III). Dow AgroSciences Study No. 160304. ABC Study No. 83625.

OCSPP 850.4100 Terrestrial Plant Tier II (Seedling Emergence) **ABC Labs**

Batch ID:	01-3170-5805	**Test Type:**	Seedling Emergence Tier II	**Analyst:**	
Start Date:	19 Feb-16	**Protocol:**	OCSPP 850.4100 Plant Seedling Emergen	**Diluent:**	Deionized Water
Ending Date:	18 Mar-16	**Species:**	Chenopodium album	**Brine:**	
Duration:	28d 0h	**Source:**	Johnny's Selected Seeds, ME	**Age:**	
Sample ID:	18-7629-8655	**Code:**	49903203	**Client:**	CDM Smith - E. Krupka
Sample Date:	19 Feb-16	**Material:**	2,4-D choline salt	**Project:**	
Receive Date:	10 Jun-16 11:07	**Source:**	Dow AgroSciences		
Sample Age:	NA	**Station:**			

Batch Note: MRID 49903203
Sample Note: MRID: 49903203

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
09-2787-9893	Mean Height	0.088	0.176	0.1245	NA		Jonckheere-Terpstra Step-Down Test
04-4463-9326	Mean Height	0.36	0.72	0.5091	50.5%		Dunnett Multiple Comparison Test
14-2078-3697	Mean Weight	0.088	0.176	0.1245	NA		Jonckheere-Terpstra Step-Down Test
07-9998-6869	Mean Weight	0.36	0.72	0.5091	78.4%		Dunnett Multiple Comparison Test
05-2337-8659	Percent Emerged	1.42	>1.42	NA	69.0%		Dunnett Multiple Comparison Test
03-4772-6760	Percent Survived	1.42	>1.42	NA	73.6%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	lbs ai/A	95% LCL	95% UCL	TU	Method
18-6236-2241	Mean Weight	IC5	0.0328	0.00899	0.0815		Nonlinear Regression
		IC10	0.0445	0.0149	0.105		
		IC25	0.101	0.0482	0.205		
		IC50	0.392	0.221	0.777		
07-1867-2071	Percent Emerged	EC5	0.0000000	N/A	N/A		Linear Regression (MLE)
		EC10	0.0000000	N/A	2.22E-07		
		EC25	0.0000001	N/A	2.26E-05		
		EC50	0.000578	N/A	0.00465		
11-1385-0186	Percent Emerged	EC50	0.938	0.664	1.33		Trimmed Spearman-Kärber
17-2876-4925	Percent Survived	EC5	0.0000000	N/A	N/A		Linear Regression (MLE)
		EC10	0.0000000	N/A	1.78E-07		
		EC25	0.0000000	N/A	0.000019		
		EC50	0.00046	N/A	0.0041		
18-3275-2551	Percent Survived	EC50	1.01	0.839	1.22		Trimmed Spearman-Kärber

OCSPP 850.4100 Terrestrial Plant Tier II (Seedling Emergence) **ABC Labs**

Mean Height Summary

C-lbs ai/A	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	5	99.8	53.7	146	66	157	16.6	37.2	37.2%	0.0%
0.00138		6	102	85.5	119	86	122	6.56	16.1	15.7%	-2.54%
0.0027		5	141	103	178	108	173	13.4	30	21.4%	-40.9%
0.0055		5	101	46.6	155	40	154	19.4	43.5	43.2%	-0.8%
0.011		6	145	112	179	108	201	13	31.8	21.9%	-45.6%
0.0221		6	118	73.2	162	66	187	17.2	42.2	35.9%	-17.7%
0.044		5	82.2	47.8	117	43	119	12.4	27.7	33.8%	17.6%
0.088		5	79.8	56.1	104	59	103	8.55	19.1	23.9%	20.0%
0.176		6	60.5	36.2	84.8	38	95	9.45	23.1	38.2%	39.4%
0.36		5	61.6	28	95.2	42	109	12.1	27.1	44.0%	38.3%
0.72		6	51.2	37.9	64.4	29	68	5.16	12.6	24.7%	48.7%
1.42		4	47.8	0.683	94.8	12	74	14.8	29.6	61.9%	52.2%

Mean Weight Summary

C-lbs ai/A	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	5	0.196	0.0559	0.336	0.0864	0.381	0.0504	0.113	57.6%	0.0%
0.00138		6	0.193	0.124	0.262	0.148	0.311	0.0268	0.0656	34.0%	1.44%
0.0027		5	0.326	0.13	0.522	0.163	0.56	0.0707	0.158	48.5%	-66.5%
0.0055		5	0.19	0.046	0.334	0.0369	0.318	0.0519	0.116	61.1%	2.99%
0.011		6	0.321	0.209	0.433	0.18	0.461	0.0435	0.107	33.2%	-64.1%
0.0221		6	0.204	0.0801	0.328	0.0925	0.405	0.0483	0.118	57.9%	-4.34%
0.044		5	0.146	0.0587	0.234	0.0661	0.244	0.0315	0.0704	48.2%	25.4%
0.088		5	0.136	0.0848	0.186	0.098	0.19	0.0183	0.0408	30.1%	30.8%
0.176		6	0.0771	0.0135	0.141	0.0204	0.178	0.0247	0.0606	78.7%	60.6%
0.36		5	0.0747	-0.00074	0.15	0.021	0.18	0.0272	0.0608	81.3%	61.8%
0.72		6	0.031	0.0116	0.0504	0.00993	0.0588	0.00755	0.0185	59.6%	84.2%
1.42		4	0.0657	-0.0149	0.146	0.0034	0.124	0.0253	0.0506	77.1%	66.5%

Percent Emerged Summary

C-lbs ai/A	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	6	0.429	0.0867	0.77	0	0.714	0.133	0.326	76.0%	0.0%
0.00138		6	0.5	0.343	0.657	0.286	0.714	0.0612	0.15	30.0%	-16.7%
0.0027		6	0.357	0.0767	0.638	0	0.714	0.109	0.267	74.8%	16.7%
0.0055		6	0.357	0.13	0.585	0	0.571	0.0884	0.217	60.7%	16.7%
0.011		6	0.381	0.155	0.607	0.143	0.714	0.0878	0.215	56.5%	11.1%
0.0221		6	0.548	0.348	0.747	0.286	0.857	0.0775	0.19	34.7%	-27.8%
0.044		6	0.429	0.239	0.618	0.143	0.571	0.0738	0.181	42.2%	0.0%
0.088		6	0.262	0.0866	0.437	0	0.429	0.0682	0.167	63.8%	38.9%
0.176		6	0.452	0.34	0.565	0.286	0.571	0.0439	0.108	23.8%	-5.56%
0.36		6	0.286	0.0737	0.498	0	0.571	0.0825	0.202	70.7%	33.3%
0.72		6	0.333	0.152	0.515	0.143	0.571	0.0706	0.173	51.9%	22.2%
1.42		6	0.143	0.00877	0.277	0	0.286	0.0522	0.128	89.4%	66.7%

Percent Survived Summary

C-lbs ai/A	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	6	0.405	0.0844	0.725	0	0.714	0.125	0.305	75.4%	0.0%
0.00138		6	0.5	0.343	0.657	0.286	0.714	0.0612	0.15	30.0%	-23.5%
0.0027		6	0.357	0.0767	0.638	0	0.714	0.109	0.267	74.8%	11.8%
0.0055		6	0.357	0.13	0.585	0	0.571	0.0884	0.217	60.7%	11.8%
0.011		6	0.357	0.13	0.585	0.143	0.714	0.0884	0.217	60.7%	11.8%
0.0221		6	0.548	0.348	0.747	0.286	0.857	0.0775	0.19	34.7%	-35.3%
0.044		6	0.405	0.165	0.645	0	0.571	0.0934	0.229	56.5%	0.0%
0.088		6	0.262	0.0866	0.437	0	0.429	0.0682	0.167	63.8%	35.3%
0.176		6	0.452	0.34	0.565	0.286	0.571	0.0439	0.108	23.8%	-11.8%
0.36		6	0.286	0.0737	0.498	0	0.571	0.0825	0.202	70.7%	29.4%
0.72		6	0.333	0.152	0.515	0.143	0.571	0.0706	0.173	51.9%	17.6%
1.42		6	0.143	0.00877	0.277	0	0.286	0.0522	0.128	89.4%	64.7%

OCSPP 850.4100 Terrestrial Plant Tier II (Seedling Emergence) **ABC Labs**

Mean Height Detail

C-lbs ai/A	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Negative Control	66	113	157		70	93
0.00138		86	122	94	89	101	122
0.0027		108	118		173	133	171
0.0055		114	40		77	118	154
0.011		142	128	158	201	108	135
0.0221		110	187	123	85	66	134
0.044		79	76	94	43		119
0.088		97	59	103	69	71	
0.176		38	57	81	54	38	95
0.36		56	42		109	47	54
0.72		53	68	54	49	54	29
1.42			74	70	12	35	

Mean Weight Detail

C-lbs ai/A	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Negative Control	0.0864	0.209	0.381		0.138	0.164
0.00138		0.166	0.148	0.154	0.15	0.23	0.311
0.0027		0.163	0.222		0.56	0.282	0.404
0.0055		0.2	0.0369		0.114	0.318	0.281
0.011		0.427	0.267	0.33	0.461	0.18	0.264
0.0221		0.152	0.405	0.234	0.0925	0.0956	0.246
0.044		0.0923	0.152	0.176	0.0661		0.244
0.088		0.168	0.109	0.19	0.113	0.098	
0.176		0.0204	0.0425	0.117	0.075	0.0298	0.178
0.36		0.0603	0.021		0.18	0.0531	0.0598
0.72		0.0302	0.0458	0.0264	0.0151	0.0588	0.00993
1.42			0.124	0.0829	0.0034	0.0528	

Percent Emerged Detail

C-lbs ai/A	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Negative Control	0.143	0.714	0.286	0	0.714	0.714
0.00138		0.429	0.571	0.429	0.571	0.714	0.286
0.0027		0.714	0.571	0	0.143	0.429	0.286
0.0055		0.286	0.286	0	0.571	0.571	0.429
0.011		0.143	0.429	0.429	0.143	0.714	0.429
0.0221		0.857	0.286	0.571	0.571	0.571	0.429
0.044		0.429	0.571	0.571	0.286	0.143	0.571
0.088		0.143	0.429	0.429	0.286	0.286	0
0.176		0.429	0.429	0.286	0.429	0.571	0.571
0.36		0.286	0.571	0	0.429	0.286	0.143
0.72		0.143	0.571	0.286	0.143	0.429	0.429
1.42		0	0.143	0.286	0.143	0.286	0

OCSPP 850.4100 Terrestrial Plant Tier II (Seedling Emergence) **ABC Labs**

Percent Survived Detail

C-lbs ai/A	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Negative Control	0.143	0.571	0.286	0	0.714	0.714
0.00138		0.429	0.571	0.429	0.571	0.714	0.286
0.0027		0.714	0.571	0	0.143	0.429	0.286
0.0055		0.286	0.286	0	0.571	0.571	0.429
0.011		0.143	0.429	0.286	0.143	0.714	0.429
0.0221		0.857	0.286	0.571	0.571	0.571	0.429
0.044		0.429	0.571	0.571	0.286	0	0.571
0.088		0.143	0.429	0.429	0.286	0.286	0
0.176		0.429	0.429	0.286	0.429	0.571	0.571
0.36		0.286	0.571	0	0.429	0.286	0.143
0.72		0.143	0.571	0.286	0.143	0.429	0.429
1.42		0	0.143	0.286	0.143	0.286	0